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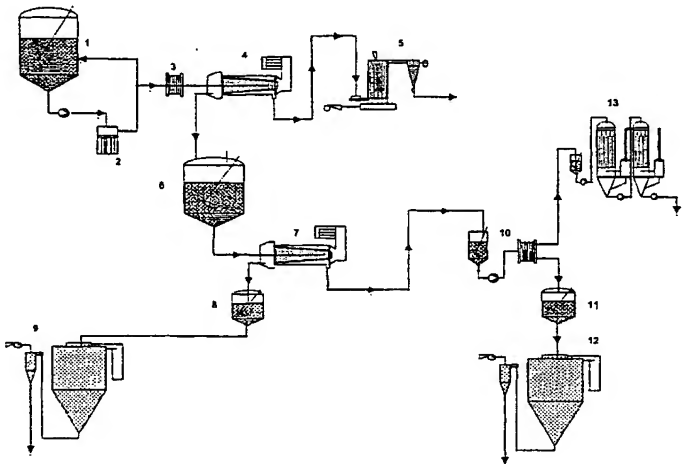
(54) Title: PROCESS FOR THE FRACTIONATION OF CEREAL BRANS



(57) Abstract: A process for the fractionation of valuable fractions from cereal brans (e.g. wheat, barley and oat brans, and rice polish) is described. In particular, this invention describes a two step process, in which the said bran is first subjected to a combination of enzymatic treatment and wet milling, followed by sequential centrifugation and ultrafiltration, which aims at physically separating the main bran factions, i.e. insoluble phase (pericarp and aleurone layer), germ-rich fraction, residual endosperm fraction and soluble sugars. A second step consists of fractionating cereal brans substantially free of soluble compounds, hence insoluble phase from the above-mentioned first step, by enzymatic treatment with xylanases and/or beta-glucanase and wet milling, followed by sequential centrifugation and ultrafiltration, which aims at physically separating the main fractions, i.e. insoluble phase (remaining cell wall components), protein-rich fraction, soluble hemicellulose and oligosaccharide, and therefore maximizes the extraction rate of valuable cell wall components and aleurone cells from previously cleaned bran.

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TITLE

PROCESS FOR THE FRACTIONATION OF CEREAL BRANS

DESCRIPTION

TECHNICAL FIELD

The present invention relates to process for the extraction of soluble proteins, non-starch carbohydrates, and optionally oils from commercially available cereal bran. It also allows the production of cell wall-derived materials and less accessible proteins from cereal brans that are substantially free of soluble compounds, the compounds thus recovered as well as their use.

BACKGROUND OF THE INVENTION

Bran is defined as the seed coat of cereal grains such as wheat, barley, rye, triticale, oat or rice. Anatomically, bran comprises the outer layers of the seed, known as the pericarp-testa and an inner layer known as the aleurone layer, which is often classified as the outermost layer of the endosperm. However, from the practical point of view cereal bran is herein defined as the remaining material after the conventional milling or polishing of cereal grains and contains primarily pericarp-testa and aleurone layer components, along with the cereal germ and residual parts of the endosperm. The relative amounts of each component will depend upon the type of cereal and milling technique applied.

Within this definition, bran therefore contains all of the pericarp- testa components, the aleurone layer, the germ components including germ proteins and oils, along with a residual amount of endosperm starch, gluten and pentosans.

US patent 4,361,651 describes a process for making fermentable sugars and high protein products from grain, mainly maize. In this method, grain is steeped for 10-30 h, prior to milling and separation of the germ component, saccharification of carbohydrates (mainly starch), and separation of fibre. The yield of starch is maximised for fermentation to alcohol. Within the described process there is no

specific fractionation of the bran component, separation of protein types or consideration of the germ component.

US patent 5,312,636 discloses a process for fractionating crop into industrial raw material. This is focused on oat grain and incorporates bran fractionation procedures that involve the extraction of more hydrophobic components such as lipids in polar organic solvents prior to the alkaline extraction of residual bran to produce beta-glucan, protein and degummed fibres. The use of the organic solvent is a key step in the process and hydrolysing enzymes are not utilised during the fractionation procedure.

Two related US patents (4,171,383 and 4,171,384) disclose dry and wet milling procedures for refining whole wheat grain. US-A-4,171,383 focuses on wet milling of the whole kernel. The bran produced is mixed with a separated (mainly) endosperm protein fraction to produce animal feed. US-A-4,171,384 describes dry milling of the whole kernel to produce an endosperm fraction, a germ fraction and a bran fraction. The endosperm fraction is then subjected to wet milling and separation of starch-rich and protein-rich fractions. The protein rich fraction is added to the bran to produce an animal feed. There is no description of a specific wet fractionation of the bran itself within either patent.

Patent application WO 99/11672 discloses a process that uses selective enzymes, such as acetyl xylan esterase and ferulic acid esterase, to both facilitate the removal of hemicellulose from various plant materials and alter its degree of phenolic ester substitution. Despite the fact that functional hemicellulose with high solubility and gelling strength can be produced yields are rather low. In fact, the inventors reported a 3 and 6% yield of arabinoxylan ferulate (hemicellulose), when wheat bran was treated with acetyl xylan esterase for 90 and 180 min, respectively. Furthermore, the invention does not make any reference to the use of xylanases, or its combination with wet milling in order to overcome the low yields reported.

US-A- 5,308,618 discloses a process to extract soluble dietary fibre hemicelluloses from wheat bran by applying a heat pre-treatment in aqueous solution. Further processing such as filtration, salting out, dialysis, ultrafiltration, reverse osmosis, gel filtration and precipitation in order to remove contaminants from the hemicellulose fraction, follows this. The inventors make no claims with regards to the use of enzymes and production of products streams other than hemicellulose. Furthermore, the invention highlights the need of run costly procedures to remove contaminants, which were once present in the original wheat bran. The bran is extracted at high temperatures and pressures in water (180 - 200°C), producing a glucose rich dietary fibre component in the water phase. The process specifically targets the production of dietary fibre and is not really / strictly a fractionation procedure in that other products is largely ignored.

US-A- 3,879,373 discloses a process to extract hemicelluloses from wheat bran by applying alkali treatment to dissolve hemicelluloses and other bran components followed by ethanol extraction to separate the hemicelluloses. Alkali (sodium hydroxide) extraction of hemicellulose has also been disclosed in US-A- 5,174,998 as an intermediate step to produce controlled-release compositions containing the said alkali-extracted hemicellulose and an active substance. Similar alkali-extraction procedure is disclosed in US-A- 4,927,649 to produce hemicellulose, which is then used in coating compositions containing insoluble dietary fibre.

WO 00/04053 patent application describes a chemical process using alkaline peroxide treatment to produce high yields of light coloured gelling hemicelluloses from products derived from flour, husk or bran. Another chemical extraction process of hemicellulose from wheat bran has been disclosed in WO98/31713 patent application, whereby the inventors combine a washing procedure to remove the starch fraction followed by an alkaline treatment with sodium hydroxide to extract the hemicellulose from the starch-free raw material.

It appears from above-described prior art on alkali extraction of hemicellulose that this is an old, proven and effective way to yield high quantities of soluble

hemicellulose with interesting functionalities such as gelling, dietary fibre and as an inert material for controlled-release compositions. The drawback of such technology is the associated problems of utilising chemicals. Firstly, chemicals eventually become contaminants in various product streams, and therefore require additional purification. This normally has significant cost implications. Secondly, innovative industrial processes based on chemical extraction are not always attractive from the marketing point of view, particularly in food applications.

Production of insoluble dietary fibres from oats is disclosed in US-A- 5,023,103, which describes a chemical procedure (alkali and bleaching treatment) for the production of insoluble dietary fibre with high water holding capacity and non-gritty mouth feel. A water holding capacity of 6.9 g water/g oat fibre has been reported.

Other references have disclosed processes for the extraction of proteins from cereal brans. US-A- 4,746,073 discloses a physical process to separate aleurone cell particles and pericarp-testa particles from commercial wheat bran. The process consists of milling the bran particles to a specific particle size distribution, electrostatically charge the said particles and then pass the said charged particles through a magnetic field, which separates aleurone from pericarp-testa particles. The separation is achieved by hammer-milling the bran and then subjecting the resultant particles to a physical separation regime achieve the separation. No aqueous wet processing is employed during the fractionation procedure described therein.

This is a rather different concept from the current invention, which is based upon the use of enzymes and aqueous wet milling.

Waszczynskyj et al. (1981) have proven that protein extraction rate of alkali-treated full fat wheat bran can be increased from 30% up to 38.5% when it is preceded by polysaccharidase treatment. The above-mentioned figures are significantly lower than those described in the present invention whereby up to 60% protein extraction rates were achieved without using alkali treatment. Furthermore, US 5,622,738

discloses a method to extract soluble hemicelluloses, for use as a source of dietary fibre, from various fibrous materials including cereal brans using alkali digestion followed by xylanase treatment. As in other prior art, Waszczynskyj et al made use of alkali digestion to improve extraction rates. Additionally, the residence time for the enzymatic treatment was rather long (3 to 96h), which makes the process not very attractive from a production cost point of view.

WO 01/60180 relates to process for separating oil from rice bran, whereby bran having a suitable particle size in a slurry is subjected to an enzymatic treatment, and subjecting the enzymatically treated slurry for a separation to recover an oil phase for further isolation of specific lipids. The process is carried out under alkaline conditions for a considerable time period, normally 15 hours. As no degradation of starch present, about 15% of the ingoing bran, takes place, any end product will be heavily contaminated with starch.

It is clear that the above-mentioned prior art has not succeeded to arrive at a process of cereal bran fractionation, which is both chemical-free and yields different food-grade fractions and simultaneously yield aleurone proteins, oligosaccharides and hemicelluloses, and yet produce insoluble dietary fibre from previously cleaned cereal bran, i.e. substantially free of soluble components, using xylanases and/or beta-glucanases in combination with wet milling.

SUMMARY OF THE PRESENT INVENTION

The main objectives of the present invention are to:

1. Arrive at an efficient and cost effective industrial wet process to extract and yield germ-, endosperm- and aleurone-rich fractions, glucose, soluble hemicellulose, soluble oligosaccharides, insoluble fibre, and optionally oils from cereal bran.
2. Combine the use of enzymatic treatment with wet milling to improve the efficiency of extraction and separation in an industrial process.
3. Ensure that in the fractionation process protein fractions of distinct physical properties and therefore functionalities were obtained.

4. Ensure that the intermediate fibre raw material contains the least amount of readily extractable components, hence solubles, so that contamination with the said solubles in the end products is kept to a minimum.
5. The process is carried out in such a way so that use of chemical extraction procedures are avoided and that, preferably food grade and non-genetically modified (non-GMO), xylanases and/or beta-glucanases are used in order to broaden the market opportunities for the end products.

In this description, the term cereal bran substantially free of soluble compounds or "cleaned bran" refers to any cereal bran, which has been processed, after conventional milling or polishing, by any means, so as to remove substantial amounts of soluble components, which are extracted by water or less polar solvents. The resulting material, hereafter referred to as cleaned bran, should contain rather limited amounts of soluble sugars, starch and gluten (less than 1%), but it may still contain some proteins and fats, which are less accessible and/or soluble. The cleaned bran consists primarily of cell wall components, of which hemicellulose is the most abundant.

The invention relates to methods, procedures and an industrial process for the wet-fractionation of cereal bran into two protein rich fractions, one of which contains the germ oils and related components, a fibre fraction, which also retains most of the aleurone proteins, and a sugar syrup fraction.

The invention is centred around the wet-milling of cereal bran in the presence of enzymes: a) starch degrading enzymes of the group amylases, and amyloglucosidases, and optionally b) non-starch degrading enzymes (polysaccharidases) and optionally a phytase, under appropriate conditions of temperature, i.e. from 50 to 90°C, more preferably from 50 to 75°C, and pH from 4 to 7.5. This is followed by the separation of the above listed components from aqueous suspension using mainly centrifugal separation methods. The pH when using an alpha amylase is normally around 7, and when using an amyloglucosidase

it is around 4.5. The enzymes are used normally in a cocktail comprising 200 to 1500 IU/g of substrate, but should contain at least 1 IU/g of substrate.

This invention also relates to methods, procedures and an industrial process for the wet-fractionation of cleaned bran into one protein rich fraction, which contains primarily proteins from the aleurone cells, a soluble hemicellulose fraction, a soluble oligosaccharide fraction and an insoluble fibre fraction.

This invention further aims at the fractionation of cleaned bran by combining wet-milling and enzymatic hydrolysis specifically with food grade xylanases and/or beta-glucanase under well-controlled conditions of temperature, such as i.e. from 35 to 80°C, more preferably from 40 to 50°C, and pH from 4 to 7, preferably 4.5 to 5.5.

Nowhere during the degradation of the bran and bran components pH exceeds 7.5 as alkaline hydrolysis seems to have a detrimental effect on the fractionation and the end products.

A further effect of the present invention is that the end products will contain no, or substantially no starch, starch derivates, or starch fragments due to the primary hydrolysis using a starch hydrolysing enzyme treatment.

This step is followed by the separation of insoluble fibre and protein fractions from aqueous suspension using centrifugal separation methods while both hemicellulose and oligosaccharide fractions are separated by size exclusion techniques such as ultrafiltration.

Presently there exist no commercial enzyme-based methods for wet-fractionating of cereal bran, which is capable of extracting the above-mentioned fractions.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

It has now surprisingly been shown possible to solve the problems identified above and meet the objectives by means of the present invention which is characterized

in that bran is first subjected to a combination of enzymatic treatment with enzymes of the group starch- and optionally phytate-hydrolysing enzymes, and aqueous wet milling, followed by an optional step of enzyme inactivation by wet heat treatment, and a subsequent step whereby the insoluble phase containing a cleaned bran consisting of both pericarp and aleurone fractions are separated by centrifugal forces into an aqueous phase containing a germ-rich fraction and an aqueous phase containing residual endosperm components, and that the proteins contained in the endosperm-rich fraction are concentrated.

In a preferred embodiment cereal brans are the fibrous-residue resulting from a primary grain milling, i.e. after the separation of the endosperm fraction, of wheat, rice, barley, oat, rye and triticale, and having variable chemical compositions, presence of anti-nutritive factors, and presence of various anatomical fractions, i.e. pericarp, germ, and residual endosperm.

In a preferred embodiment the enzymatic treatment is accomplished using a starch degrading enzyme in the form of a polysaccharidase of amylases and/or amyloglucosidases.

In a preferred embodiment a further enzymatic treatment is carried out using at least one non-starch degradable polysaccharidase in the form of cellulases, hemicellulases mainly xylanases, beta-glucanases, and pectinases, and/or phytases.

In a preferred embodiment such cleaned bran is subjected to a combination of enzymatic treatment with specific enzymes of the group xylanase and/or beta-glucanase under strictly controlled hydrolysis conditions, and intermittent wet milling, followed by an optional step of enzyme inactivation by wet heat treatment.

In a preferred embodiment the inactivated hydrolysate is then fractionated by centrifugal forces into an insoluble phase containing primarily cellulose, lignin, less accessible hemicellulose, residual aleurone cells and cell wall bound proteins, and

an aqueous phase containing soluble hemicellulose, oligosaccharides, sugars and proteins, and that the aqueous phase is further separated by centrifugal force into protein-rich fraction and a carbohydrate-rich fraction, and that the carbohydrate-rich fraction is further separated by size exclusion technique into a hemicellulose-rich fraction (medium molecular size) and an oligosaccharide-rich fraction (small molecular size).

In a preferred embodiment cereal bran substantially free of both in water or less polar solvents soluble compounds are derived from wheat, rice, barley, oat, rye or triticale.

In a preferred embodiment the combination of intermittent wet milling with enzymatic treatment is arranged to increase substrate accessibility to the cell wall degrading enzymes thereby improving the overall hydrolysis performance and the subsequent separation of the various fractions by density/solubility and molecular size.

In a preferred embodiment the enzymatic treatment is carried out using at least one non-starch degradable polysaccharidase in the form of cellulases, hemicellulases mainly xylanases, beta-glucanases, and pectinases, and optionally phytases.

In a preferred embodiment the enzymatic treatment is accomplished by using xylanases with high beta 1-4- xylanase (pentosanase) and/or beta-glucanase activity.

In a preferred embodiment the said fraction contains at least 35% protein and 10% oil on dry matter basis and exhibits a high emulsifying capacity and an increased shelf life with regards to resistance to oxidation compared to the original bran, and that the said fraction contains less than 5% fibre.

In a preferred embodiment the said fraction contains at least 25% protein and 10% sugar and less than 3% oil and 3% fibre, and at least 25% soluble high-molecular

weight non-starch polysaccharides of the groups beta-glucans for barley and oat and arabinoxylans for wheat, rice, rye and triticale.

In a preferred embodiment liquid whey is incorporated in to the said fraction at levels varying from 20 to 80% by weight on dry matter basis, and that the final mixture is dried.

A further aspect of the invention comprises an insoluble fibre fraction produced wherein the said fraction consists of cell wall components of bran (>85%) and aleurone proteins (>10%), and substantially free of gluten and starch, and with a high water holding capacity (>6g water/g dry product).

A still further aspect of the invention encompasses a sugar fraction wherein the said fraction is originated primarily from the residual endosperm and it contains more than 65% sugars (such as glucose, maltose and maltotriose) on dry matter basis.

A further aspect of the invention encompasses a protein fraction derived substantially from the aleurone cells, wherein the said fraction contains at least 35% protein and 10% oil, less than 5% insoluble fibre on dry matter basis, substantially free of gluten and starch and with a high emulsifying capacity.

A still further aspect of the invention encompasses an insoluble fibre fraction, wherein the said fraction consists primarily of cell wall components with a relative lower hemicellulose content compared to the original cleaned cereal bran, substantially free of gluten and starch (<1% on dry matter basis) and with a high water holding capacity (>6g water/g dry product).

A further aspect of the invention relates to a soluble hemicellulose fraction, wherein the said fraction consists primarily of medium molecular weight hemicellulose preferably above 20kDa (>40%) of the groups arabinoxylans from wheat, rye, rice and triticale, and beta-glucans from oat and barley, which also contains proteins